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Influence of Cultural Factors on Alfalfa Seedling Infection by *Pythium debaryanum* Hesse

By W. F. Buchholtz

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IOWA STATE COLLEGE OF AGRICULTURE AND MECHANIC ARTS

BOTANY AND PLANT PATHOLOGY SECTION

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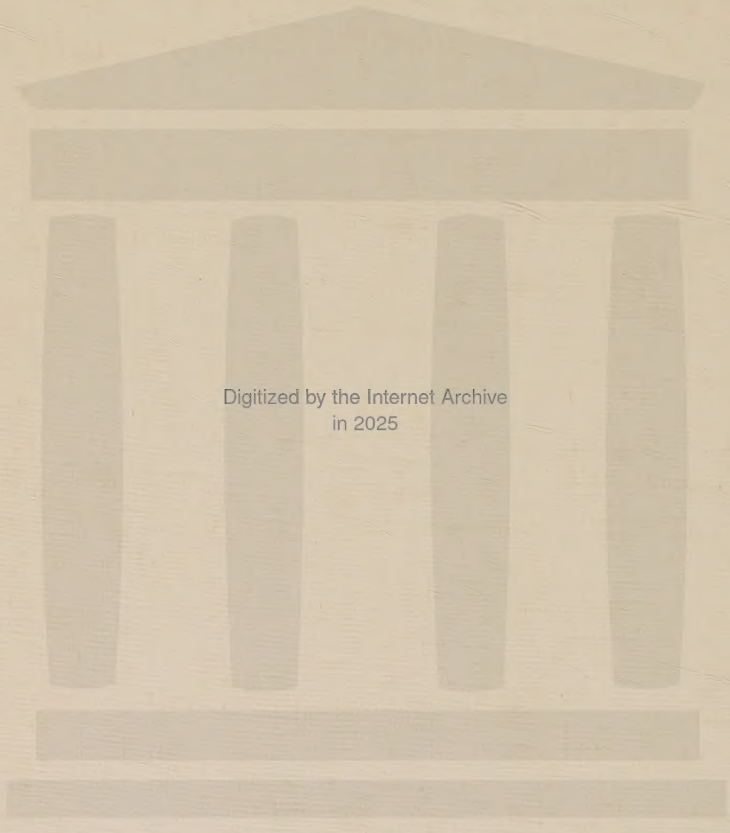

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SUMMARY

Pythium debaryanum Hesse was shown to cause rapid direct or indirect necrosis of germinating seed or seedlings of alfalfa and to retard development of seedlings by primary and secondary root girdling and root tip necrosis. Such activity by *P. debaryanum* resulted in partial or occasionally complete losses of seedling stands and in dwarfed plants with subnormal root systems inadequate to carry the plants through periods of high temperature and low soil moisture.

Infection of alfalfa seedlings by *P. debaryanum* tended to be more prevalent in acid Iowa soils (pH value below 6.6) than in neutral soils (pH value above 6.6), although during the hot dry summer of 1934, some soils in central, southern and southeastern Iowa apparently were free of *P. debaryanum*. At the same time the pathogen was present in the soils of northern Iowa where there was some surface soil moisture.

Lime was ineffective as a control for *P. debaryanum* in laboratory experiments but yielded small increases in seedling stands on acid soil in field plots at Kanawha in 1935. Liming resulted in small but inconsistent increases in stand at Kanawha in 1937 and 1938.

Seed treatment with ethyl mercuric phosphate was an effective control measure in laboratory plantings and in some field plantings in 1935. In subsequent field plantings seed treatment did not result in increased seedling stands.

Early plantings usually yielded better stands than did later spring plantings.

Deep plowing was an effective control measure in 1935, but produced smaller and less consistent results in 1937 and 1938. *Pythium debaryanum* appeared to be more abundant in the surface soil than in soil at depths below 4 inches. Deep plowing turned up relatively uninfested soil for the seedbed.

Red clover, alsike clover and white Dutch clover appeared more resistant to *P. debaryanum* than alfalfa in greenhouse plantings in infested soil. Red clover and alsike yielded much better stands than alfalfa in unplowed, fallow, infested soil in single plot field plantings in 1935. Sweet clover stands in the same plantings were also better than the alfalfa stands.

These studies indicate that it may be possible to improve alfalfa seedling stands by planting early, to a limited extent by liming acid soil and by deep plowing to expose uninfested soil from depths below 4 inches for the seedbed.

Influence of Cultural Factors on Alfalfa Seedling Infection by *Pythium debaryanum* Hesse

By W. F. Buchholtz[2]

It is well recognized that considerable care must be exercised on some soils to establish stands of small-seeded legumes, notably alfalfa and red clover and sometimes sweet clover. Farmers generally are aware of the necessity of sowing a good grade of seed of a hardy variety on a well-prepared seedbed under a light nurse crop, after the soil, if acid, has been limed. The common rate of seeding alfalfa is 10-15 pounds per acre, which, with good-quality seed, provides for 50-75 seeds per square foot. Second year stands in Iowa, however, rarely contain more than 10 plants per square foot. Obviously a reduction of any part of an alfalfa stand loss, with its accompanying hazard of complete failure, would be of real benefit to the Iowa farmer.

The role of Pythium in destroying alfalfa seedling stands on three acid Iowa soils was first pointed out by the author (1, 2) in two short preliminary papers in 1934 and 1935. Pythium was observed to produce necrotic lesions on germinating seed and alfalfa seedlings more commonly on slightly acid Clarion loam, Webster loam and Tama silt loam than on two similar areas of neutral Webster loam and Webster silty clay loam. At 9°C. fewer alfalfa and alsike clover seedlings became infected than at 20°-25° C. Plantings of alfalfa and alsike clover on steamed and formaldehyde-treated acid soil yielded as many or more healthy alfalfa seedlings than similar plantings on neutral field soil. Alsike seedlings seemed more resistant to Pythium than alfalfa seedlings at 20°-25° C. The prevalence of infection of alfalfa seedlings in acid soils at moderate (15°-30° C.) temperatures is comparable to the situation recently reported (3) from sugar beet seedling damping-off studies in northern Iowa.

In 1935 Grandfield, Lefebvre and Metzger (5) reported alfalfa stand failures on fallowed soils in Kansas as a result of damping-off. The greatest reduction in stand and plant vigor occurred on plots fallowed 3 years or more. Steam, dry heat and formaldehyde treatment of the soil partially controlled the pathogen. Isolations from necrotic lesions of diseased seedlings yielded cultures of Fusarium, Helminthosporium, Trichoderma

[1] The major portion of this bulletin comprises part of a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree, Doctor of Philosophy. Project 432 of the Iowa Agricultural Experiment Station.

[2] The author gratefully acknowledges the helpful suggestions offered by Dr. I. E. Melhus during the course of these investigations and his critical reading of the manuscript. Thanks are due also to Miss Marie Corkle who prepared figs. 1 and 2.

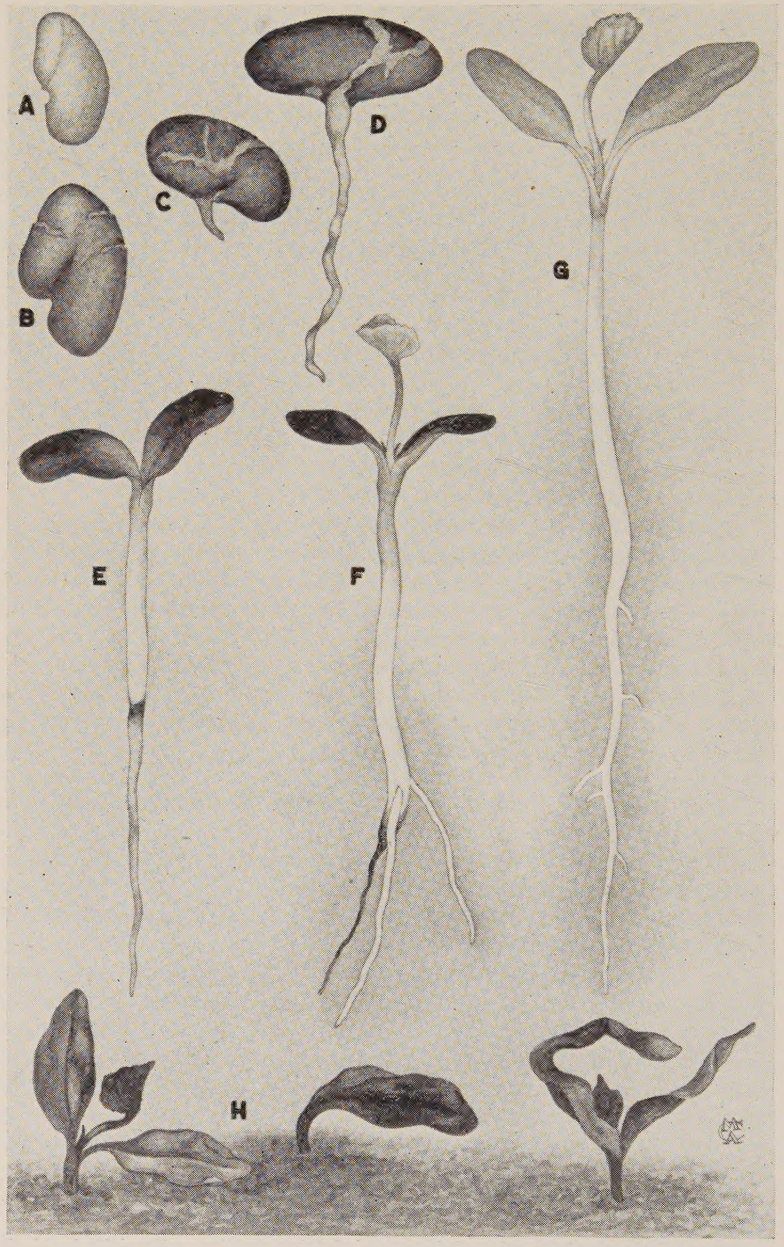

Fig. 1. (Legend on opposite page)

and Pythium. Isolates of Pythium predominated, and they only were pathogenic.

The purpose of this bulletin is to present further evidence which suggests that the stand and vigor of alfalfa seedlings may be appreciably reduced by the soil-borne seedling and root parasite, *Pythium debaryanum* Hesse, and that an understanding of its biology in the soil may aid in defining procedures most likely to result in the successful establishment of alfalfa stands.

DAMPING-OFF SYMPTOMS

Pythium debaryanum may infect any part of the embryo of a germinating seed[3], the primary or secondary roots and hypocotyls of seedlings, and to some extent the secondary roots of older plants.

GERMINATING SEED

The entire embryo may be infected and reduced to a brown gelatinous mass while within the seed coat, or the radicle and cotyledons may become brown and softened outside the seed coat (fig. 1—B, C, D). When infection occurs in later germination stages the lesions are more typically limited to the hypocotyl and root, which are temporarily water-soaked and flaccid but not at all or only slightly discolored (light brown). The tissues of such lesions collapse quickly and in a day or two undergo brown discoloration and complete disintegration. In soils heavily infested with *Pythium debaryanum*, 75 percent of the viable seed may fail to germinate.

SEEDLINGS

Hypocotyl and root infections of seedlings also result in water-soaked, flaccid and finally collapsed lesions (fig. 1—E, F). Brown discoloration of the hypocotyl near the soil line is somewhat more rapid than that of the germinating seed. Severe lesions on the primary root or hypocotyl results in stunted, flaccid, abnormally dark green cotyledons, and with high temperatures and low humidity, in wilting, desiccation and death of

[3] In this discussion germination of alfalfa seed is considered complete when the cotyledons are expanded and the first secondary root develops. In soil in the presence of light, completion of germination thus coincides with "emergence" above the soil line.

Fig. 1. Symptoms of alfalfa seed decay and seedling damping-off caused by **Pythium debaryanum.** A. A dry unplanted seed. B. An infected germinating seed in which the contents were reduced to a brown gelatinous mass by the fungus. C, D. Germinating seeds with visibly collapsed, brownish, discolored radicles. Portions of the cotyledons within the seed coat were softened and brown. E. Infected seedling showing typical discoloration and necrosis of the hypocotyl and primary root. F. Seedling with only brown shrunken remains of the primary root. The secondary roots that arose above the necrotic zone are healthy, and one of them seems to be assuming the position of the tap root. G. A healthy seedling. H. Diseased alfalfa seedlings as they appear in the field. The cotyledons, leaves and hypocotyls collapse in 6 to 24 hours on a windy, warm day. The cotyledons of diseased seedlings turn yellow, purple and finally become flaccid and brown. In cooler weather the diseased seedlings linger, becoming dark green and stunted.

the entire seedling, sometimes within 6 to 24 hours (fig. 1—H). On the other hand, with a humid atmosphere, moderate temperature and sufficient soil moisture, a seedling with a diseased primary root or hypocotyl may survive by virtue of secondary roots which arise under such conditions from uninfected tissue above the lesion (fig. 1—F). Later some of these secondary roots also may become diseased and in turn develop secondary roots. Young roots have been observed on which there was evidence of three successive developments of secondary roots, each above lesions. The process frequently results in a branched root system replacing at least temporarily the normal tap root structure common to alfalfa.

OLDER PLANTS

As the primary and first secondary roots become older, infection commonly results in discoloration and disintegration of only the cortex, leaving the central vascular cylinder exposed and temporarily rough and shrunken, a typical "wire root." Such cortical lesions become inconspicuous when the root thickens.

Plants with infected roots, but with the vascular cylinder of the primary root intact, exhibit a curling, yellowing, wilting and dying of the cotyledons, lower leaves, and only occasionally of the entire plant. Sometimes such plants are merely smaller and less vigorous in general appearance than plants with an uninfected root system. They may persist for some time, a few perhaps as long as healthy plants, but their impaired root system must limit their development under highly competitive circumstances.

THE CAUSAL AGENT

When the loss of alfalfa stands through seedling damping-off was first recognized and reported in 1934 (1), isolations had been made that pointed to members of the genus Pythium as the cause of the disease. Not enough information was available to enable an assignment of isolates to species. It was fundamentally significant to determine whether one or more species were involved, and if only one were involved, to observe its characteristics and their variation within and between isolates.

ISOLATION RESULTS

Water-soaked, non-collapsed lesions resulting from apparently recent infections of very young alfalfa seedlings consistently yielded cultures of *Pythium debaryanum*. One washing and at least three thorough rinses with sterile, distilled water were found necessary to eliminate troublesome bacterial contamination. If present in the tissues of a lesion, *P. debaryanum* grew

out to form a colony covering a petri dish of potato-dextrose agar in 24 to 36 hours at room temperature. A petri dish of plain agar usually was covered by the colony in 36 to 72 hours at room temperature. Plain agar was useful in establishing bacteria-free cultures.

In this investigation *P. debaryanum* was first successfully cultured from alfalfa on potato-dextrose agar on June 16, 1934, when it was taken from 16 of 26 diseased seedlings. On June 19, 29 of 40 and on June 21, 9 of 16 diseased seedlings yielded *P. debaryanum*; 8 healthy seedlings yielded none. These were seedlings grown on Clarion and Webster loam at Kanawha, Hancock County. On Aug. 7, 25 of 34 diseased seedlings grown on Tama silt loam, State Center, Marshall County, and 7 of 12 from Webster loam, Ames, yielded *P. debaryanum*. Six of these isolates taken at random produced damping-off of alfalfa seedlings in steamed soil in the greenhouse and were recovered from their respective diseased seedlings. In 1936, 50 fungous isolates were taken from diseased alfalfa seedlings grown at Kanawha. Forty-three were *P. debaryanum*, three were unidentified Phycomycetes, three *Fusarium* sp. and one *Alternaria* sp. Twenty of the 43 *P. debaryanum* isolates were tested for pathogenicity to alfalfa seedlings; all were pathogenic. A series of isolations from alfalfa seedlings grown on Grundy silt loam in southern Iowa in 1937 yielded *P. debaryanum* as follows: On May 6, 15 of 20 from Indianola, Warren County, and 9 of 9 from Lamoni, Decatur County; on May 8, 10 of 10 from Lamoni; on May 15, 10 of 10 from Oskaloosa, Mahaska County; on June 2, 11 of 20 from Oskaloosa. Each isolate was pathogenic to alfalfa seedlings. All the evidence from isolations indicates clearly that *P. debaryanum* was the primary cause of the damping-off of alfalfa seedlings in 1934, 1936 and 1937. No isolations were made in 1935.

IDENTITY OF ISOLATES

Assignment of isolates to the species *Pythium debaryanum* Hesse was made only after their characteristics had been compared with those of a representative group of 20 isolates that were studied in detail. These 20 were taken at random from 43 isolates of *P. debaryanum* obtained from alfalfa in 1936. Although not identical, they exhibited basic similarities as follows: Single, smooth oogonia, either terminal or intercalary, were formed in 3 to 5 days on plain agar and a small cube of steamed carrot in a petri dish. Usually one antheridium was associated with each oogonium, on a stalk arising from the same hypha at least 10μ from the base of the oogonium or oogonial stalk (fig. 2—H-L). Conjugation took place by the passage of the antheridial contents into the oogonium. The point of contact of the

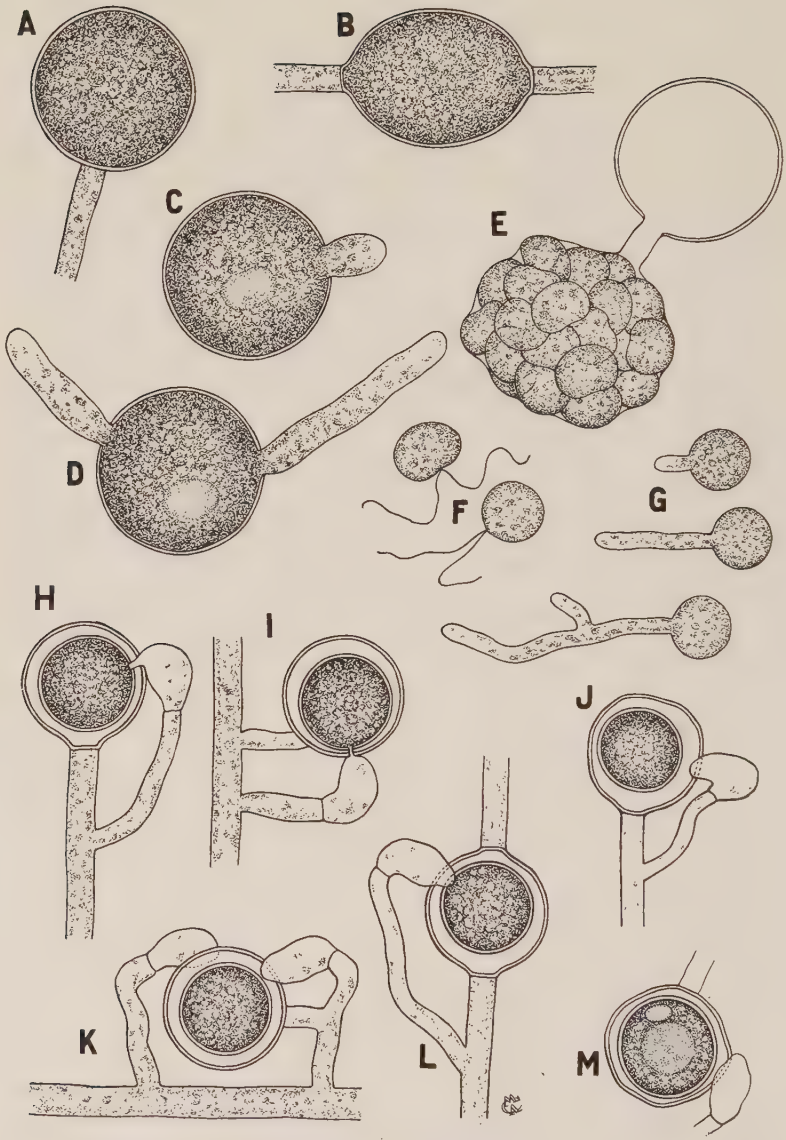

Fig. 2. Sporangia, zoospores, antheridia and oogonia, and oospores of **Pythium debaryanum.** A, B. Terminal and intercalary sporangium, respectively. C, D. Germination of sporangia by germ tubes. E. Germination of sporangium by formation of zoospores in a vesicle; zoospores approaching state of motility. F. Motile biciliate zoospores. G. Zoospores germinating by germ tubes after coming to rest. H-L. Antheridia and oogonia with developing oospores; H, J and L show most typical disposition of the antheridium. M. Oogonium with mature oospore. All x 1000.

antheridium and oogonium usually was constricted, and the antheridial stalk was entirely free from the oogonium or the oogonial stalk. Later, one or two other antheridia, rarely more, might approach the oogonium. The second and third antheridia commonly were attached to other nearby hyphae. Diameters of 180 oogonia were measured: 12 were 14μ; 51, 17μ; 92, 19μ; 21, 21μ; and 4, 24μ. The average diameter was 18.44μ (table 1). Average oogonial diameters of individual isolates ranged from 17.3μ (isolate 106) to 19.4μ (isolate 160) (table 1). The smooth, light brown oospores did not fill the oogonium (fig. 2—M) and were from 12 to 19μ in diameter with an average of 15.4μ. Isolates differed in the relative number of oogonia produced. One isolate, No. 106, produced sporangia and oogonia in abundance; other isolates produced many sporangia, but few oogonia. Though sparse, the oogonia produced by such isolates were typical. Oospore germination was not observed.

Sporangia were either terminal on long or short hyphal branches or intercalary (fig. 2—A, B) in cultures on a boiled hemp seed in distilled water in a petri dish. Sporangia were larger and more variable in size than oogonia. Measurements of 180 sporangia were as follows: 3 were 14μ; 5, 17μ; 13, 19μ; 21, 21μ; 64, 24μ; 48, 26μ; 20,29μ; 3, 31μ; 2, 34μ, and 1, 39μ (table 1). The average diameter was 24.32μ and averages of individual isolates ranged from 21.4μ (isolate 106) to 26.6μ (isolate 145). Occasionally zoospore formation was observed on a boiled hemp seed culture in distilled water as follows (fig 2—E, F): The sporangial protoplast flowed out through an opening in the sporangial wall and rounded up to form a thin-walled vesicle.

TABLE 1. DIAMETERS IN MICRONS OF OOGONIA AND SPORANGIA OF 20 ISOLATES OF PYTHIUM DEBARYANUM.

Isolate No.	Oogonia diameters						Sporangia diameters									
	14μ	17μ	19μ	21μ	24μ	Av.	14μ	17μ	19μ	21μ	24μ	26μ	29μ	31μ	34μ	Av.
104	2	1	4	2	..	18.1	..	..	...	2	1	5	1	...	...	25.0
105	2	2	4	1	...	17.7	1	..	1	..	6	1	..	..	...	22.6
106	1	5	3	...	..	17.3	..	2	2	1	2	2	..	...	...	21.4
118	2	3	2	2	..	17.7	...	1	1	...	4	3	...	...	...	23.3
119	..	5	3	1	..	18.1	...	1	1	...	1	3	2	...	1	25.6
120	...	4	5	..	..	18.1	..	1	...	2	4	1	...	1	...	23.6
121	2	...	6	1	...	18.1	...	..	...	...	4	2	3	...	..	26.1
130	..	4	4	1	..	18.3	1	...	1	1	4	1	..	...	1	23.3
131	1	3	4	...	1	18.3	...	..	1	1	5	2	...	...	...	23.6
133	..	2	5	2	...	19.0	...	...	1	2	4	2	..	...	..	23.2
134	...	2	6	1	..	18.8	..	...	1	...	4	3	1	...	..	24.7
145	...	3	6	...	..	18.3	...	..	...	3	1	1	3	...	*	26.6
146	...	3	5	1	..	18.5	..	...	1	...	2	5	1	...	..	25.1
157	..	4	4	1	...	18.3	...	...	1	1	2	5	..	...	..	24.2
158	1	1	5	2	...	18.7	...	..	1	...	5	1	1	1	...	25.0
159	...	2	6	1	...	18.8	..	...	...	2	2	3	1	1	...	25.3
160	..	3	4	...	2	19.4	1	..	..	...	3	4	1	..	...	24.3
357	1	...	6	1	1	19.2	...	..	...	3	2	1	3	...	...	24.9
378	..	2	5	2	...	19.0	...	...	1	1	4	2	1	..	...	24.1
379	..	2	5	2	..	19.0	...	..	...	2	4	1	2	...	...	24.8
Totals	12	51	92	21	4	18.44	3	5	13	21	64	48	20	3	2	24.32

*One sporangium 39μ in diameter.

Soon, cleavage lines became evident with subsequent breaking up of the protoplast into individual masses, which quickly became motile. The activity of the now biciliate, kidney-shaped zoospores eventually disrupted the vesicle membrane and they swam away. They remained active for a short time, after which they rounded up and germinated by a germ tube (fig. 2—G). Sporangia germinated directly by a germ tube (fig. 2—C, D.) when transferred to malt extract.

According to the method of zoospore production by sporangia, the position of the oogonium and its accompanying antheridium or antheridia, and the diameters of oogonia, oospores and sporangia, all of the 20 isolates examined fell within the limits of *P. debaryanum* Hesse as interpreted by Drechsler (4) and redescribed by Matthews (7). All other isolates reported as *P. debaryanum* also fell within these limits, except that zoospore production was not observed in all isolates.

PATHOGENICITY OF *PYTHIUM DEBARYANUM* TO ALFALFA UNDER VARIOUS CLIMATIC AND CULTURAL CONDITIONS IN THE FIELD AND LABORATORY

In preliminary trials and early observations it was noted that soil acidity, soil temperature, soil depth and to a certain extent soil moisture influenced the pathogenicity and apparent abundance of *Pythium debaryanum*. From greenhouse and field experiments designed to measure such influences was taken the evidence which follows. Data from greenhouse experiments are presented first.

DAMPING-OFF OF ALFALFA SEEDLINGS IN SOIL SAMPLES COLLECTED FROM DIFFERENT PARTS OF IOWA, 1934

Among the first observations made were those at three locations, one near Ames and one at Kanawha, both on Clarion and Webster loam, the other near State Center on Tama silt loam. More diseased alfalfa seedlings were observed in plantings on acid soil than in similar plantings on nearly neutral soil. It seemed worthwhile, therefore, to survey the state to see whether *Pythium debaryanum* was destructive to alfalfa seedlings in acid soils from other parts of Iowa.

At various times from early April until late September, 1934, 50 soil samples were taken in various parts of Iowa as shown in fig. 3. For each sample a clean, quart Mason jar was filled with surface (0-3 inches) soil from two places not less than 3 and not more than 6 feet apart in what was considered to be a typical soil situation for the field. If the field was distinctly uneven in topography, one sample was taken from a typical upland area and another from a typical lower area not far distant and always

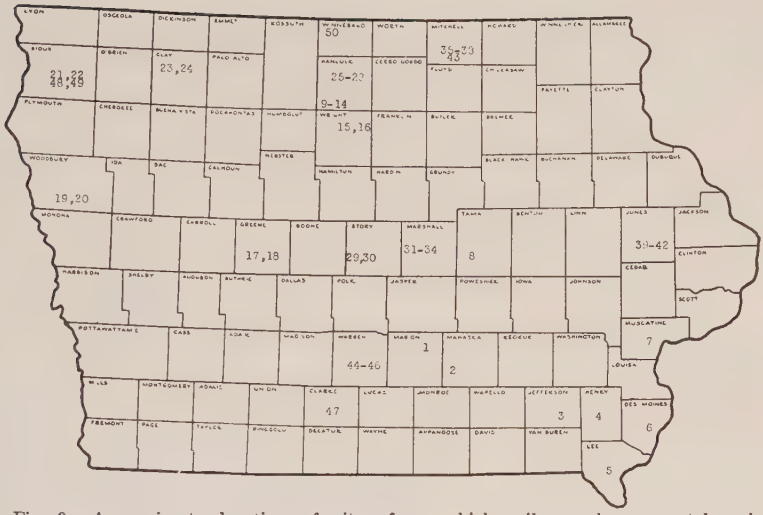

Fig. 3. Approximate location of sites from which soil samples were taken in 1934. (The numbers are serial numbers of the 50 samples and correspond to the "sample no." of table 2.)

either supporting the same crop or under the same tillage conditions. The sample jar was covered with a Mason lid, without a rubber seal, and stored in the refigerator at 5° C. until collection of all samples had been completed, at which time half of each sample was placed in each of two washed, 4-inch pots, and two rows of 50 Grimm alfalfa seeds each were planted in each pot. In 2 weeks, when germination was complete and in some pots diseased seedlings were abundant, healthy and diseased seedlings were counted. In the meantime colorimetric pH determinations had been made in duplicate for each sample. The results are presented in table 2.

The summer of 1934 was very hot, and in southern Iowa extremely dry[4]. In general, the samples from central, southern and eastern Iowa were collected when the weather had been hot and dry for some time; samples from north central and northwest Iowa were collected during more moderate conditions. The data are separated under these two categories in table 2. Since the results were not anticipated, no moisture content determinations were made.

[4] In the general summary of the Iowa Weather and Crop Bureau these statements appear: "The average temperature for the state of Iowa for the year 1934 was 3.6° above the 62-year average—the third warmest of the 62 years of record. All months except September and December were warmer than normal, and May—was the warmest May of record. Days with 100° or higher averaged 21, about twice the greatest number heretofore recorded in any year; the largest number was 42 at Lenox (Taylor County, southern Iowa). Most stations of the southern and western portions of the state established new monthly and seasonal high temperature records.

"Precipitation was deficient for 9 consecutive months ending with June. Water shortage was serious in the southern and western portions of the state—because of excessive, prolonged heat, scorching sunshine and increased wind movement—."

TABLE 2. NUMBER OF HEALTHY AND DISEASED ALFALFA SEEDLINGS GROWN FROM 200 SEEDS IN THE GREENHOUSE ON SOIL SAMPLES COLLECTED IN IOWA DURING 1934. (APPROXIMATE SITE OF COLLECTION OF EACH SAMPLE IS SHOWN IN FIG. 2.)

Soil pH value below 6.6			Soil pH value above 6.6			
Seedlings			Seedlings			
Sample No.	Healthy	Diseased	Sample No.	Healthy	Diseased	
1	134	19	5	152	0	
2	147	0	18	160	1	
3	176	0	28	159	0	
4	117	25	30	158	2	
6	155	6	....	.....	...	
7	145	0	.....	.....	...	
8	133	7	.....	.....	...	Collected during low soil moisture and high temperature conditions from central, southern and southeastern Iowa
17	151	0	....	.....	...	
27	158	0	....	.....	...	
29	113	7	.....	.....	...	
31	127	44	.....	.....	..	
32a	50	81	.....	.....	...	
33	163	0	.....	.....	...	
34	164	2	.....	.....	...	
39	162	0	.....	.....	..	
40	159	5	....	.....	...	
41	151	12	.....	.....	..	
42	171	0	.....	.....	..	
44	133	1	.....	.....	..	
45	136	7	.....	.....	...	
46	127	0	....	.....	...	
47	59	56	.....	.....	...	
Av.	137.7	12.3	.....	157.2	0.7	
9	11	62	10	112	22	Collected from north central and northwestern Iowa during moderate soil moisture and temperature conditions except where indicated
11	141	3	12	152	0	
13	37	40	14	115	27	
20	42	89	15	147	0	
21	36	84	16	158	0	
22	58	88	19	167	1	
23	107	28	24	85	66	
35b	161	0	25	113	52	
36b	154	16	26	109	30	
37b	138	1	50	86	17	
38b	142	22	....	.....	....	
43a	13	51	.....	.....	.....	
Av.	86.6	40.3	.....	124.4	21.5	

a From low spot.
b Very dry.

Among central and northern Iowa soil samples, damping-off of alfalfa seedlings was in general more common in the soil samples with a pH value below 6.6 than in the samples with a pH value above 6.6. This tendency was less evident in samples collected in southern Iowa during hot, dry weather. In cases where there are large numbers in the "diseased" column the number of healthy seedlings is more significant, since considerable interruption of germination occurred.

Subsequently-collected moist samples from the same areas as dry samples 29, 30, 31 and 32 were tested in the manner previously described. The resulting data are presented in table 3 alongside those previously taken for the similar dry samples. In every case the moist samples yielded fewer healthy and more diseased seedlings than the similar dry samples collected pre-

TABLE 3. NUMBER OF HEALTHY AND DISEASED ALFALFA SEEDLINGS GROWN FROM 200 SEEDS IN THE GREENHOUSE ON DRY AND MOIST SOIL SAMPLES COLLECTED AT DIFFERENT TIMES FROM THE SAME PLACES. (APPROXIMATE SITE OF COLLECTION OF EACH SAMPLE IS SHOWN IN FIG. 2.)

Sample No.	pH	Collected on June 28 during hot dry weather.		Collected in October after period of rainfall and moderate temperature.	
		SEEDLINGS		SEEDLINGS	
		Healthy	Diseased	Healthy	Diseased
29	6.1	113	7	31	105
30	7.2	158	2	154	17
31	6.0	127	44	22	88
32	5.8	50	81	15	101

viously. There were fewer diseased seedlings in moist sample 30 (pH 7.2) than in the three acid (pH 5.8-6.1) moist samples. There is reason to believe, therefore, that the lack of *P. debaryanum* in the dry, acid samples from southern and southeastern Iowa was not indicative of their condition under more nearly average soil moisture and temperature conditions. On the contrary, these results indicate a lethal effect of low moisture and high temperature on *P. debaryanum* in the soil. A similar effect was detected in Kansas by Grandfield, Lefebvre and Metzger (5).

DAMPING-OFF OF ALFALFA SEEDLINGS ON LIMED AND UNLIMED SOILS IN THE GREENHOUSE

An apparent association of the prevalence of *Pythium debaryanum* and its destructiveness to alfalfa with soil acidity and the success of the common practice of adding lime to correct soil acidity for the establishment of legume stands suggested that at least a partial effect of lime might be control of *P. debaryanum*. Preliminary experiments with excessive amounts of lime indicated such a possibility. To obtain more evidence the following greenhouse experiments were performed. In the first experiment eight pots filled with unsteamed acid Clarion loam from Kanawha were limed with calcium carbonate at the rate of 10 tons per acre. In each of these and in eight check pots of unlimed soil were planted immediately one row each of 25 treated and 25 untreated alfalfa seeds. The average numbers of healthy and diseased seedlings were as follows:

	Unlimed		Limed	
	Healthy	Diseased	Healthy	Diseased
Untreated	12	6	12	6
Treated	18	3	19	2

It is clear that the liming was without effect.

In the second experiment, acid soil samples were collected as follows: Clarion loam from Kanawha and Ames, Carrington silt loam from Osage, and Buckner fine sand from Conesville, with pH 6.4, 6.2, 5.7, and 5.3, respectively. Lime was added to half of each sample to meet the lime requirement as shown by the Emerson test. Two-thirds of each half was then steamed, and half of each steamed lot was infested with a pure culture of *P. debaryanum*. Six 3-inch pots were filled with each sub-sample so that there were 36 pots of each original sample. Ten alfalfa seeds were planted in each pot, and the plantings watered daily. Remaining seedlings were counted after 30 days. The results appear in table 4. With the exception of the contrast between stands on infested and uninfested soils, none of the differences was convincingly large, and the significant fact was that in this experiment there was no marked increase in seedling stand from liming either in the unsteamed soil or the steamed-infested soil.

TABLE 4. NUMBER OF ALFALFA SEEDLINGS FROM 60 SEEDS PLANTED ON THREE ACID IOWA SOILS, STEAMED AND UNSTEAMED, LIMED AND UNLIMED, INFESTED AND NOT INFESTED WITH PYTHIUM DEBARYANUM.

Soil type	pH	Unsteamed soil		Steamed soil			
				Uninfested		Infested	
		Unlimed	Limed	Unlimed	Limed	Unlimed	Limed
Clarion loam Kanawha	6.4	28	27	42	33	2	0
Ames	6.2	31	36	40	33	4	1
Carrington silt loam Osage	5.7	39	25	43	39	10	6
Buckner fine sand Conesville	5.3	21	24	27	22	3	1

DAMPING-OFF OF ALFALFA SEEDLINGS IN SOIL SAMPLES COLLECTED AT DIFFERENT DEPTHS

In culture, *Pythium debaryanum* manifests a somewhat aerobic nature, especially in its tendency to form mycelial mats at the surface of water cultures. Johnson (6) reports that in sand to which different amounts of water had been added, *P. debaryanum* grew abundantly at or just above the level of the water in the sand. In the light of such a phenomenon, it seemed reasonable that in a compact moist soil *P. debaryanum* might decrease in abundance at successively lower levels. That such may sometimes be the case is substantiated by the ensuing observations and experiments.

Late in the summer of 1934 there were no diseased seedlings in alfalfa plantings in samples of Clarion loam collected at 18-

and 36-inch depths from a Kanawha plot, while plantings in surface (0-3 inches) samples yielded only a 34-percent stand of healthy seedlings. In October, 1-quart samples of soil were collected at 2-inch intervals down to 8 inches in two locations on Clarion loam at Kanawha, one location on Clarion loam at Ames and one location on Tama silt loam near State Center. The number of healthy and diseased alfalfa seedlings from two planted rows of 50 alfalfa seeds in each of two pots in the greenhouse, together with pH and lime requirement determinations for each sample, appear in table 5. In each of the four locations there was a decided tendency for the surface soil to yield fewer healthy and more diseased seedlings than samples from successively deeper layers. In the Kanawha collections most of the diseased seedlings were in samples from a depth of 6 inches or less, while in the other two cases they were in samples taken above a depth of 4 inches. It was difficult to keep the sub-surface samples entirely free of the small soil particles which escaped the sampling tools in removing surface samples, so that there was some possibility of contamination. It seems reasonable to conclude that the surface soil samples (upper 4 inches) were much more heavily infested with *P. debaryanum* than the samples taken below 4 to 6 inches.

The fact that the surface soil was heavily infested and subsurface soil only lightly infested with *P. debaryanum* suggested the possibility that turning up large quantities of sub-surface soil by deep plowing might afford a relatively non-infested seedbed. This possibility was further upheld by the fact that in a four-row planting at Kanawha on carefully turned over infested

TABLE 5. NUMBER OF HEALTHY AND DISEASED ALFALFA SEEDLINGS GROWN FROM 200 SEEDS IN THE GREENHOUSE IN SOIL SAMPLES COLLECTED AT DIFFERENT DEPTHS FROM THREE DIFFERENT SOIL AREAS, OCTOBER, 1934.

Soil	Depth, inches	Lime req. tons per A.	pH	Seedlings	
				Healthy	Diseased
Acid Clarion loam (Kanawha)	0-2	1.5	6.3	1	105
	2-4	1.25	6.2	27	94
	4-6	1.5	6.2	11	80
	6-8	1.0	6.4	158	12
(2nd series of samples)	0-2	1.5	6.1	1	56
	2-4	1.75	6.1	9	84
	4-6	1.5	6.0	43	73
	6-8	1.0	6.0	155	29
Acid Clarion loam (Ames)	0-2	2.0	6.0	43	84
	2-4	2.0	6.1	13	67
	4-6	2.0	6.1	105	40
	6-8	1.0	6.3	148	16
Acid Tama silt loam	0-2	2.0	6.4	64	70
	2-4	1.5	6.4	62	75
	4-6	1.0	6.2	127	33
	6-8	1.0	6.5	159	7

soil (top 3 inches and next 3 inches of soil carefully interchanged) there was an average of 251 seedlings per row as contrasted to 124 seedlings per row in a comparable planting on adjacent undisturbed infested soil. Further evidence on plowing effects, to be presented under "field results," tend to substantiate this experience.

SEED TREATMENT[5]

Seed treatment resulted in increased alfalfa stands in soil infested with *Pythium debaryanum* in several greenhouse and field experiments. The following greenhouse experiment is typical and indicates also the relative susceptibility to *P. debaryanum* of alfalfa, alsike, white Dutch clover and red clover. One row of 25 treated and 1 row of 25 untreated seeds of alfalfa, alsike clover, white Dutch clover and red clover were planted in each of four pots of naturally infested acid Clarion loam and two pots of steamed Clarion loam. Mean numbers of healthy and diseased seedlings per row appear in table 6. With all the legumes except white Dutch clover on unsteamed soil there were more healthy and fewer diseased seedlings from treated than from untreated seed. On steamed soil there were no diseased seedlings from treated or untreated seed and practically no difference in the number of healthy seedlings from treated and untreated seed. The failure of alsike, white Dutch clover and red clover to become infected by *P. debaryanum* as abundantly as alfalfa on infested acid soil suggests that the "acid-tolerance" of red clover, alsike and white Dutch clover may be associated with resistance to *P. debaryanum*. This relationship held also in the field in 1935 and will be discussed further when the 1935 field data are presented.

In other greenhouse experiments, similar in part to the one described above, stand differences attributable to seed treatment were like those just enumerated. No positive results from seed treatment were obtained after 1935. In plantings of 100 seeds per row of treated and untreated alfalfa and red clover seed replicated 4 times on 3 dates at 11 locations in Iowa in 1937, there were no consistent seedling stand increases from seed treatment. In a seed treatment experiment with 12 fungicides on Clarion loam at Kanawha in 1938, no consistent increases occurred with 5 replications of 500-seeds-per-row plantings. All the field soil involved in greenhouse and field experiments up to and including 1935 was heavily infested with *P. debaryanum*. It is quite possible that after 1935 the heavy infestation disappeared on the Kanawha plot and that such a heavy infestation as occur-

[5] In all cases, unless otherwise specified, seed treatment was with 5 percent ethyl mercuric phosphate, as much as adhered to the seed when the excess fungicide was screened off.

red at Kanawha up to 1935 was not encountered in any subsequent plantings. Under conditions of limited infestation the slight toxic effect of the fungicide might partially nullify the limited protective effect.

Seed treatment cannot be recommended as a partial control in view of its failure to result consistently in increased seedling stands and its probable interference with commonly practiced methods of legume seed inoculation.

TABLE 6. MEAN NUMBERS OF HEALTHY AND DISEASED SEEDLINGS OF ALFALFA, ALSIKE, WHITE DUTCH CLOVER AND RED CLOVER GROWN IN THE GREENHOUSE FROM TREATED AND UNTREATED LOTS OF 25 SEEDS ON STEAMED AND UNSTEAMED ACID CLARION LOAM.

	Unsteamed Clarion loam				Steamed Clarion loam			
	Untr. seed		Tr. seed		Untr. seed		Tr. seed	
	Heal.	Dis.	Heal.	Dis.	Heal.	Dis.	Heal.	Dis.
Alfalfa	14.5	6.5	19.7	0.5	21.5	0	20.5	0
Alsike	16.7	2.2	20.2	1.2	23	0	23	0
Red clover	14.2	2.2	18.2	0	18.5	0	17	0
White Dutch clover	20.7	0	18.7	0	19	0	18.5	0

FIELD RESULTS

Preliminary experiments indicated that: 1. alfalfa damping-off was less severe at 9° C. than at 25° C., 2. *Pythium debaryanum* was less abundant at soil levels below a depth of 4 inches, which fact might be important in plowing, and 3. that such "acid-tolerant" legumes as red clover, alsike and white Dutch clover might be somewhat resistant to *P. debaryanum.*

Field plantings were made in 1935, 1937 and 1938 of alfalfa, sweet clover, red clover and alsike clover at different dates, on limed and unlimed soil, plowed and unplowed. It was not feasible to include all the enumerated variables in every plot, but in several experiments more than one were included. The results of such individual experiments are presented in their entirety and the relative effect of the variables compared. The effect of seed treatment was an aid in estimating interrupted germination, an indirect measure of *P. debaryanum* infestation of the soil.

STANDS OF ALFALFA, SWEET CLOVER, RED CLOVER AND ALSIKE CLOVER ON PLOWED AND UNPLOWED, LIMED AND UNLIMED, ACID AND NEUTRAL SOILS IN 1935.

In the spring of 1935 there was available at Kanawha a set of comparable situations as follows:

Plot 1. Acid (pH 6.0) Clarion loam, fallowed since 1932, unlimed.

2. Acid (pH 6.0) Clarion loam, fallowed since 1932, limed.
3. Neutral (pH 6.8-7.0) Webster silt loam, fallowed since 1932, unlimed.
4. Neutral (pH 6.8-7.0) Webster silt loam, fallowed since 1932, limed.
5. Acid (pH 6.0) Clarion loam, cornland 1934, spring plowed, 1935.
6. Neutral (pH 6.8-7.0) Webster silt loam, cornland 1934, spring plowed, 1935.

Plots 1, 2 and 5 were adjacent and 3 and 4 were adjacent. Plot 6 was 50 rods from 3 and 4 but identical in soil type and nearly so in topography. On each of these plots were planted on two dates, May 6 and May 29, 4 rows 5 ft. long of untreated and 4 rows of treated alfalfa seed, 4 rows of untreated white blossom sweet clover seed, 4 rows of untreated red clover seed and 4 rows of untreated alsike seed. Each row was planted with 500 seeds. The 500 seeds were uniformly distributed in the bottom of furrows cleaned, smoothed and covered by hand. Both plantings were preceded and shortly followed by approximately 1.5 inches rainfall. Average soil temperature during the germination period was 12.0° C. for the first planting and 17.5° C. for the second planting. Total seedlings were counted when germination was completed. In plots with poor stands, seedlings of all four legumes revealed typical symptoms of infection by *P. debaryanum*. The mean numbers of seedlings per row in each plot are presented in table 7.

The data allow comparisons of stand differences between plantings, between plowed and unplowed soil, between acid and neutral soil, limed and unlimed, and between legumes. The following discussion will enumerate these differences in more detail and suggest possible interpretations. Differences in alfalfa stands will be considered first.

DIFFERENCES IN ALFALFA SEEDLING STANDS

There were large and consistent differences in alfalfa seedling stands between plowed and unplowed plots. Both acid and neutral plowed cornland (plots 5 and 6) yielded much better stands of alfalfa seedlings than the similar fallowed areas (plots 1 and 3).[6]

Seed treatment resulted in greater seedling stands in all cases in the fallowed plots where the stands from untreated seed were low, but was relatively ineffective in plowed ground.

Liming the soil resulted in more seedlings on acid Clarion loam, but slightly fewer on neutral soil.

[6] There is no proof here that the previous cropping history might not have contributed to the difference except that "turned over" (previously recorded) fallow soil yielded slightly better stands (251.0 seedlings per row) at the time of the second planting than the plowed Clarion loam cornland (plot 5, 235.0 seedlings per row).

TABLE 7. MEAN SEEDLING STANDS PER ROW FROM 500 SEEDS OF ALFALFA, WHITE SWEET CLOVER, RED CLOVER AND ALSIKE CLOVER ON PLOWED AND UNPLOWED, LIMED AND UNLIMED SOIL, KANAWHA, 1935.

Soil type, condition and cultural treatment	Alfalfa				Sweet Clover		Red Clover		Alsike	
	1st planting (May 6)		2nd planting (May 29)							
	Untreated seed	Treated seed	Untreated seed	Treated seed	1st planting	2nd planting	1st planting	2nd planting	1st planting	2nd planting
FALLOW SINCE 1932						(All from untreated seed.)				
Acid Clarion loam										
Unlimed (1)*	51.0	132.5	124.2	201.7	180.0	229.5	265.7	258.5	293.0	350.7
Limed (2)	136.7	211.7	148.7	260.0	239.2	255.7	276.0	261.0	355.0	411.0
Neutral Webster silt loam										
Unlimed (3)	105.2	164.2	170.0	241.0	225.0	245.5	243.2	258.7	310.5	398.7
Limed (4)	94.7	146.7	111.7	225.2	208.2	248.5	248.7	248.5	334.7	357.5
PLOWED CORNLAND										
Acid Clarion loam (5)	270.7	291.7	235.0	283.5	329.5	300.2	335.7	291.0	442.5	381.2
Neutral Webster silt loam (6)	345.0	307.5	314.0	297.2	355.2	332.5	365.2	318.0	424.5	409.7

*"Plot numbers" in text.

Differences between first and second plantings were consistent but in different directions on plowed and fallow soil. In each of eight comparisons there were more seedlings from the second planting on fallow soil than from the first (plots 1, 2, 3, 4).[7] In each of four comparisons on plowed soil (plots 5, 6) there were fewer seedlings in the second planting. It seems reasonable that *Pythium debaryanum* might have been slowly reinvading the relatively non-infested soil turned up by plowing; it also might be possible that the *P. debaryanum* infestation was declining somewhat in the fallow soil as the season progressed.

RELATIVE DIFFERENCES BETWEEN STANDS OF ALFALFA, WHITE BLOSSOM SWEET CLOVER, RED CLOVER AND ALSIKE ON FALLOW AND ON PLOWED PLOTS

Seedings of alsike clover, red clover and even sweet clover sometimes succeed when seedings of alfalfa fail under the same circumstances, particularly on acid soil. Red clover and alsike are considered more "acid-tolerant" than alfalfa or sweet clover. In a previous discussion of greenhouse results with seed treatment it was pointed out that in field soils infested with *Pythium debaryanum*, stands of white Dutch clover, another "acid-tolerant" legume, alsike and red clover appeared not to be heavily infected. To test this possibility in the field, alsike; red clover; and sweet clover, the other common small-seeded "lime-requiring" legume, were planted alongside alfalfa in the 6 plots previously described. Counts of seedling stands of these legumes appear in table 7.

Differences in alfalfa stands on the various plots were largely the result of differences in germination caused by *P. debaryanum*. It is reasonable to expect, therefore, that the germination of similarly susceptible species might be interrupted by *P. debaryanum* in the same plots in the same proportion. Conversely, it is probable that seeds of resistant species would germinate better than those of susceptible species in low-stand plots and little or no better in high-stand plots. When the data are examined such a relationship is indeed evident. Alfalfa stands from untreated seed were very low in fallow plots as compared with alfalfa stands on plowed plots. Stands of sweet clover, alsike and red clover, on the other hand, were much better than alfalfa stands from untreated seed on the fallow plots and little or no better on the plowed plots, particularly on plowed Webster silt loam (plot 6). These comparisons, plus the evidence presented in table 6 and observations of other greenhouse experiments not herein recorded, would seem to indicate a somewhat greater susceptibility of alfalfa as compared with red clover, alsike and white Dutch clover. The data in

[7] A still earlier planting of alfalfa (April 20) on fallow acid soil (plot 1) yielded 75.5 seedlings per row of untreated seed and 160.2 seedlings per row of treated seed, which is more than from the May 6 planting, fewer than from the May 29 planting.

table 7 do not permit a clear differentiation between sweet clover, red clover and alsike. None was immune. Infected seedlings of red clover, alsike and sweet clover were not difficult to find in the fallow plots.

ALFALFA STANDS FROM TWO BROADCAST PLANTINGS ON LIMED AND UNLIMED, PLOWED AND UNPLOWED ACID SOIL IN 1937 AND 1938

In 1936 the center half of the Kanawha plot was plowed, and it was used in its entirety for sugar beet experiments. In 1937 the two outside fourths were plowed and the center half left unplowed so that two pairs of adjacent plowed and unplowed plots crossed eight alternating strips, four limed and four unlimed. There were 32 plots in all. Two half-rod-square broadcast plantings at 14 pounds per acre, one with treated and one with untreated seed, were made on each plot at two dates, May 18 and June 9 and 10. Wet soil prevented earlier plantings. Stand counts at three times during the season are shown in table 8 as plants per square foot. There was no difference in alfalfa seedling stand from seed treatment in this experiment in 1938; therefore, data from treated and untreated plots have been averaged and are thus presented.

TABLE 8. ALFALFA PLANTS PER SQUARE FOOT FROM BROADCAST PLANTINGS AT TWO DATES ON PLOWED AND UNPLOWED LIMED AND UNLIMED ACID SOIL AT KANAWHA, 1937.

		First planting (May 18)			Second planting (June 9, 10)		
		June 4	July 20	Oct. 15	June 30	July 20	Oct. 15
Unplowed	Unlimed	34.0	33.3	14.9	26.8	23.7	11.3
	Limed	33.5	31.7	15.0	30.0	26.8	12.0
Plowed	Unlimed	36.2	32.7	15.0	29.0	23.4	12.8
	Limed	40.7	38.1	16.2	32.5	25.8	13.9
Averages	Unplowed	33.7	32.9	14.9	28.3	25.2	11.6
	Plowed	38.4	35.4	15.6	30.7	24.6	13.3
	Unlimed	35.1	33.0	14.9	27.8	23.6	12.0
	Limed	37.1	35.3	15.6	31.2	26.3	12.9
	Total	36.08	34.12	15.26	29.55	24.89	12.49

The comparison of plantings on plowed and unplowed, and limed and unlimed plots was repeated on the same plot at Kanawha in 1938. The area unplowed in 1937 was plowed and the area plowed in 1937 was left unplowed in 1938. A "square" planting technique was devised to reduce the plot size and better control the number of seeds planted per area counted, as follows:

A single "square" consisted of an area of exactly 10 square feet divided into quarters, 2½ square feet to each quarter. In each quarter were planted 100 alfalfa seeds together with 50-65 seeds of oats for a nurse crop, so that in each 10 square feet

were planted 400 seeds of alfalfa and 200-250 seeds of oats. At each of two dates two such squares were planted on each soil plot. In other respects the arrangement of the plots was the same as in 1937, except that two limed and one unlimed strips were removed on one side of the plot leaving three limed alternating with two unlimed strips.

TABLE 9. ALFALFA PLANTS PER SQUARE FOOT FROM BROADCAST PLANTINGS ON TWO DATES ON PLOWED AND UNPLOWED LIMED AND UNLIMED ACID SOIL AT KANAWHA, 1938.

		First planting (April 23, 24)			Second planting (May 19)		
		May 11	June 17	Aug. 19	June 17	July 9, 11	Aug. 19
Unplowed	Unlimed	19.7	17.7	6.0	15.8	12.1	6.2
	Limed	22.1	18.8	6.4	16.3	12.1	6.8
Plowed	Unlimed	20.6	19.1	7.1	19.2	16.2	6.6
	Limed	21.9	20.4	7.5	20.2	17.8	7.6
Averages	Unplowed	20.9	18.2	6.2	16.1	12.1	6.5
	Plowed	21.2	19.7	7.3	19.6	17.0	7.1
	Unlimed	20.2	18.4	6.5	17.5	14.1	6.4
	Limed	22.0	19.6	7.0	18.2	14.9	7.2
	Total	21.1	19.0	6.8	17.9	14.5	6.8

Both plantings were counted three times, immediately after germination, about a month later and on Aug. 19. The data obtained, expressed as plants per square foot, are presented in table 9. Second and third counts are not very reliable because one replication of plowed plots was badly washed.

The data from the two experiments show that in these two seasons an increase in seedling stands was consistently obtained by planting alfalfa early. Smaller and less consistent increases in seedling stands were obtained by plowing the soil and by liming.

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